Bactericidal properties of inorganic acids against mycobacteria

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Abstract

Despite current successes in fighting animal tuberculosis, sporadic cases of infection with both the disease's causative agents and atypical mycobacteria still occur in animals. Since animal mycobacteriosis cannot be treated with medication, priority must be given to their prevention and elimination as non-specific measures, including disinfection. The unique structure of the mycobacteria cell determines their high resistance to antimicrobial agents, therefore the constant search for effective disinfectants is an urgent task of veterinary science. This study aimed to determine the bactericidal properties of several inorganic acids against mycobacteria. Experiments were carried out following modern methodological aspects using the atypical mycobacteria *M. fortuitum*, pathogens of tuberculosis *M. bovis* and *M. avium*. Orthophosphoric (phosphoric) acid (H_3PO_4) and nitric acid (HNO_3) were used in the experiments. Using the suspension method, orthophosphoric acid was found to have a bactericidal effect against atypical mycobacteria *M. fortuitum* and the pathogen of tuberculosis *M. avium* when tested at a concentration of 1.5% (24 hours) and 2.0% (1–24 hours), against the causative agent of tuberculosis *M. bovis* at a concentration of 1.5% (5–24 hours) and 2.0% (1–24 hours). Nitric acid showed a bactericidal effect when tested in solution against mycobacteria *M. fortuitum* and *M. avium* at a concentration of 2.0% (5–24 hours), and against *M. bovis* at a concentration of 2.0% after 1 hour exposure. A different level of mycobacterial growth intensity in test tubes was observed after the action of inorganic acids in sub-bactericidal and bacteriostatic concentrations, which was directly dependent on the concentration and exposure of the acid, and on the type of test culture of microorganisms. It has been shown that inorganic acids can disinfect surfaces contaminated with the tuberculosis-causing agent. This refers to various surfaces like wood, tile, fabric, glass, and metal. It has been found that orthophosphoric acid (1.5% for 24 hours) and nitric acid (2.0% for 5 hours) can be used to disinfect in cases of tuberculosis infection. These findings have been confirmed through bioassays on laboratory animals. Future research will focus on discovering and developing new medications with strong bactericidal properties against mycobacteria.

Key words: *orthophosphoric acid; nitric acid; mycobacteria; concentration; exposure; test objects; bactericidal effect*

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Introduction

Existing measures to combat infectious animal diseases can be effective and efficient only if it is possible to break the epizootic chain or its links. To this end, it is necessary to increase the resistance of susceptible animals and to ensure the destruction of pathogens at all phases of their life cycle in the environment by available and effective means of mechanical, chemical, physical, and biological effects (Sander et al., 2020; Matsuzaki et al., 2021; Ghanbari et al., 2022).

Today, tuberculosis still holds a special place among zoo anthroponotic diseases. According to the world's leading tuberculosis experts, this disease remains a particularly dangerous infection for humans today (Boisson-Dupuis, 2020; Migliori et al., 2021).

Bovine tuberculosis is a serious infectious disease affecting a wide range of domesticated and wild animals, representing a global economic and public health burden (Turgenbayev et al., 2021; Borham et al., 2022). *M. bovis* is a pathogen at the human-livestock-wildlife interface. Diseases transmitted between humans, livestock, and wildlife are increasingly challenging public and veterinary health systems (Mohamed, 2019; Byrne et al., 2022).

Cattle can contract tuberculosis due to various reasons, such as feeding young animals with non-disinfected skim milk, bringing in cattle from TB positive farms and exposing them to the general herd, contact with infected cattle while grazing or drinking water, and most importantly, failure to follow proper health and anti-tuberculosis prevention measures. These measures include maintaining good sanitation on holdings, disinfecting facilities and farm territories, and using disinfected manure (Bouchez-Zacria et al., 2018; Sedighi and Varga, 2021).

Many experiments have shown that mycobacteria are highly resistant to environmental pressures, chemicals, and physical factors (Saxena et al., 2021). While sunlight can be effective in killing mycobacteria in the external environment, this outcome can be influenced by various factors. Therefore, it is essential to use disinfectants to sanitise contaminated objects (Zhang et al., 2022; Balani et al., 2023).

Various disinfectants from different groups of chemical compounds are used to neutralise mycobacteria in the environment (Bocian et al., 2014; Shinoda et al., 2016). However, when studying the properties of different disinfectants, we found that most of them do not cause the devitalisation of mycobacteria or have only a bacteriostatic effect, and only some of them have a pronounced bactericidal effect against atypical mycobacteria and tuberculosis pathogens (Zavgorodnii et al., 2021; Uy et al., 2022).

These data indicate that the arsenal of disinfectants active against the causative agents of tuberculosis and atypical mycobacteria is limited, and the search for new disinfectants is an actual and promising area of veterinary medicine.

Traditional means of disinfection are gradually losing their relevance due

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to the formation of resistance in field isolates of pathogens, so scientists are working on determining the bactericidal properties of other means used in various industries.

The aim of this study was to examine the bactericidal properties of several inorganic acids against mycobacteria.

Materials and methods

Two inorganic acids were used in the experiment: orthophosphoric (phosphoric) acid, nitric acid. Orthophosphoric acid (H_3PO_4) is an 85% aqueous solution of a syrup-like consistency that is both colourless and odourless. Nitric acid (HNO_3) is a colourless liquid with a pungent, specific smell. The mass fraction of the main substance is ≥ 56.0%.

Experiments were carried out following the current methodological recommendations for the approval of disinfectants in agriculture (Wales et al., 2021).

The bactericidal properties of the acids were determined against the tuberculosis pathogens *M. bovis* and *M. avium*, and the fast-growing atypical mycobacteria *M. fortuitum*, cultured on Pavlovsky's medium for 30-45 and 14-21 days, respectively, at a temperature of 37.0±0.5°C. The bacterial mass of mycobacteria test cultures, which had typical cultural and biological properties, was used in the experiments.

The bactericidal effect of acids against the causative agent of tuberculosis *Mycobacterium bovis* (strain *Vallee*), *Mycobacterium avium* (strain *IECVM UAAS*) and atypical mycobacteria *Mycobacterium fortuitum* (strain 122) was tested at concentrations of 0.1; 0.5; 1.0; 1.5; 2.0% at exposures of 1, 5 and 24 hours.

Before the experiment, a suspension at a concentration of 2 billion bacterial bodies per cm^3 of sterile isotonic solution was prepared from mycobacteria test cultures grown on Pavlovsky's medium. To do this, the bacterial mass of mycobacteria test cultures was transferred with a bacteriological loop into sterile vials filled with beads with a capacity of 100–200 cm^3, pre-weighed on analytical scales. The mass of mycobacteria introduced into them was determined by weighing, and then the necessary volume of sterile isotonic solution was introduced. Vials were shaken on a shuttle apparatus for 30 minutes until a homogeneous suspension of mycobacteria was obtained.

After that, working solutions of acids were prepared in the above concentrations, which were introduced in vials with a capacity of 20 cm^3 (10 cm^3 in each). Then, 0.2 cm^3 of the suspension of the corresponding mycobacteria species was introduced into each test vial. The contents of the vials were thoroughly mixed and subjected to the specified exposure to the disinfectant. As a control of the bactericidal effect of the tested drug, a vial with a suspension of mycobacteria test cultures was used, in which 10 cm^3 of a sterile isotonic solution was added instead of disinfectant solutions.

Then samples of 10 cm^3 were taken from the experimental and control vials, transferred to centrifuge tubes, and centrifuged at 3000 rpm for 30 minutes.

To stop the action of the disinfectants in the test tubes, the sediment formed after centrifugation, as well as the control sample, were washed twice with a sterile isotonic solution by centrifugation.

After that, the sediment suspension was inoculated on a nutrient medium for the cultivation of mycobacteria. Test tubes with cultures were kept in a thermostat at a temperature of 37.0±0.5°C for 90 days, and after inoculation the growth of cultures was recorded every 3–5 days.

Determination of the bactericidal properties of inorganic acids was also carried out using test objects: wood, ceramic tile, batiste, glass, metal using the *Mycobacterium bovis* test culture and biological load (liquid manure).

A mixture containing 1 cm^3 of the tuberculosis pathogen test culture suspension and 0.5 cm^3 of sterile manure was applied to each test object. After that, the experimental test objects were treated with the working disinfectant solutions. A sterile isotonic solution was applied separately to the control test objects instead of the disinfectant. After the given exposure, from each control and experimental test object, scrapings and swabs with a sterile isotonic solution in Petri dishes were made. The contents were transferred to centrifuge tubes and centrifuged at 3000 rpm for 30 minutes. To neutralise the effects of the drugs the sediment in the test tubes was washed twice with sterile isotonic solution by centrifugation. The obtained sediment of experimental and control samples was resuspended in 5 cm^3 sterile isotonic solution and inoculated with a sterile pipette on a nutrient medium for the cultivation of mycobacteria. Guinea pigs were inoculated with this solution.

Test tubes with cultures were kept in a thermostat at a temperature of 37.0±0.5°C for three months, and every 3–5 days the growth of cultures was recorded.

The biological experiment was performed on 24 healthy experimental and 10 control guinea pigs weighing 300–350 g. Guinea pigs were subcutaneously injected in the groin suspension of the sediment obtained after processing the experimental and control test objects with culture *Mycobacterium bovis* at a dose of 1 cm^3.

Laboratory animals were observed for 3 months. During this period, the animals were examined once a month with a tuberculin test. Animals that died during the experiment and were sacrificed after its completion were examined by pathoanatomical and cultural methods for tuberculosis.

The experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On the Protection of Animals from Cruelty") and international bioethical norms (the materials of the IV European Convention on the Protection of Vertebrate Animals, which are used for experimental and other purposes, Strasbourg, 1985) (Simmonds, 2017).

Results and discussion

The preliminary determination of the bactericidal effect of inorganic acids was carried out against atypical mycobacteria of the species *Mycobacterium fortuitum* using the suspension method. The results of the research are presented in Table 1.

The analysis of the obtained results shows that orthophosphoric acid at exposure from 1 to 5 hours at a concentration of 0.1-0.5% does not show an antimicrobial effect against *M. fortuitum*, and the growth of mycobacterium cultures on the nutrient medium is similar to the control cultures. When the exposure was increased to 24 hours, as well as when using acid at a concentration of 1.0% at exposure of 1-5 hours and a concentration of 1.5% at exposure of 1 hour, the growth of mycobacteria on the nutrient medium decreased slightly, and amounted from 20 to 50 colonies on the surface of the nutrient medium. When exposure increased to 24 hours at a concentration of acid 1.0% and 5 hours at a concentration of 1.5%, the growth of mycobacteria decreased twice, compared to the control.

Table 1. Bactericidal effect of inorganic acids against *M. fortuitum* in solution

Mode of the acid application		Research results	
Concentration	Exposure	Experiment	Control
Orthophosphoric acid			
0.1%	1 hour 5 hours 24 hours	++++ ++++ +++	++++ ++++ ++++
0.5%	1 hour 5 hours 24 hours	++++ ++++ +++	++++ ++++ ++++
1.0%	1 hour 5 hours 24 hours	+++ +++ ++	++++ ++++ ++++
1.5%	1 hour 5 hours 24 hours	+++ ++ –	++++ ++++ ++++
2.0%	1 hour 5 hours 24 hours	– – –	++++ ++++ ++++
Nitric acid			
0.1%	1 hour 5 hours 24 hours	++++ ++++ +++	++++ ++++ ++++
0.5%	1 hour 5 hours 24 hours	++++ ++++ ++	++++ ++++ ++++
1.0%	1 hour 5 hours 24 hours	+++ +++ +	++++ ++++ ++++
1.5%	1 hour 5 hours 24 hours	+++ ++ +	++++ ++++ ++++
2.0%	1 hour 5 hours 24 hours	+ – –	++++ ++++ ++++

Note: "–" – there is no colony growth; "+" – up to 10 colonies of mycobacteria on the surface of the nutrient medium; "++" – from 10 to 20 colonies of mycobacteria on the surface of the nutrient medium; "+++" – from 20 to 50 colonies of mycobacteria on the surface of the nutrient medium; "++++" – more than 50 colonies of mycobacteria on the surface of the nutrient medium

The lack of growth of mycobacteria cultures was observed after exposure to orthophosphoric acid at a concentration of 1.5% for 24 hours and at a concentration of 2.0% and exposure from 1 to 24 hours, which indicates the manifestation of the bactericidal effect of this compound.

As for nitric acid, when tested at a concentration of 0.1-0.5% at exposure of 1-5 hours; at a concentration of 0.1% at

Table 2. Bactericidal effect of inorganic acids against *M. bovis* and *M. avium* in solution

Inorganic acid	Mode of the acid application		Research results	
	Concentration	Exposure	Experiment	Control
	Mycobacterium bovis			
Orthophosphoric acid	1.5%	1 hour 5 hours 24 hours	+ – –	++++ ++++ ++++
Orthophosphoric acid	2.0%	1 hour 5 hours 24 hours	– – –	++++ ++++ ++++
	Mycobacterium avium			
Orthophosphoric acid	1.5%	1 hour 5 hours 24 hours	++ ++ –	++++ ++++ ++++
Orthophosphoric acid	2.0%	1 hour 5 hours 24 hours	– – –	++++ ++++ ++++
	Mycobacterium bovis			
Nitric acid	2.0%	1 hour 5 hours 24 hours	– – –	++++ ++++ ++++
	Mycobacterium avium			
Nitric acid	2.0%	1 hour 5 hours 24 hours	+ – –	++++ ++++ ++++

Note: "–" – there is no colony growth; "+" – up to 10 colonies of mycobacteria on the surface of the nutrient medium; "++" – from 10 to 20 colonies of mycobacteria on the surface of the nutrient medium; "++++" – more than 50 colonies of mycobacteria on the surface of the nutrient medium

exposure of 24 hours; at a concentration of 1.0-1.5% at exposure of 1-5 hours, the growth of mycobacteria was similar to the effect of orthophosphoric acid. When using nitric acid at a concentration of 0.5% at exposure of 24 hours, the same result was obtained as when using orthophosphoric acid at a concentration of 1.0%. Under the action of nitric acid at a concentration of 1.0-1.5% at 24-hour exposure and at a concentration of 2.0% at 1-hour exposure, sporadic growth of mycobacteria cultures on the nutrient medium was observed, which indicated the manifestation of the sub-bactericidal effect of this agent. When using acid at a concentration of 2.0% at exposure from 5 to 24 hours, mycobacteria growth on the nutrient medium was absent, proving the presence of a bactericidal effect.

At the next stage, the bactericidal effect of inorganic acids against the causative agents of tuberculosis *M. bovis* and *M. avium* was studied using the suspension method, taking into account the results of previous experiments. The results of the research are presented in Table 2.

Data in Table 2 show that orthophosphoric acid at a concentration of 1.5% after exposure for 1 hour has a sub-bactericidal effect against the causative agent of tuberculosis *M. bovis*, while the growth of mycobacteria did not exceed 10 colonies on the surface of the nutrient medium. When the exposure time was increased to 5-24 hours, as well as at a concentration of 2.0% after exposure of 1-24 hours, the growth of mycobacteria on the nutrient medium was not observed. Somewhat different results were obtained in experiments with the culture of the causative agent of tuberculosis *M. avium*. For example, at an acid concentration of 1.5% at exposure of 1-5 hours, the growth of cultures was up to 20 colonies of mycobacteria on the surface of the nutrient medium. Along with this, under the action of orthophosphoric acid at a concentration of 1.5% at exposure of 24 hours and at a concentration of 2.0% at exposure from 1 to 24 hours, a bactericidal effect was observed.

In experiments with nitric acid, it was established that the culture *M. bovis* completely died under the action of acid at a concentration of 2.0% after exposure for 1-24 hours, while the causative agent *M. avium* was resistant to the action of acid after exposure for 1 hour. When the exposure was increased to 5 and 24 hours, the bactericidal effect of nitric acid was also established.

After obtaining positive results of preliminary experiments in solutions, the final determination of the mode of bactericidal action of inorganic acids was carried out against *Mycobacterium bovis* using test objects: wooden bars, ceramic tiles, batiste strips, metal and glass plates. The results of the conducted research are shown in Table 3.

Table 3 shows that orthophosphoric acid at a concentration of 1.5% after exposure for 1 hour did not disinfect test objects contaminated with *M. bovis*, the causative agent of tuberculosis, regardless of their physical properties. When the exposure was increased to 5 hours, the disinfecting effect on ceramic tiles, glass, and metal was observed, while the live causative agent of tuberculosis was isolated from wood and batiste. Similar results were obtained when using nitric acid at a concentration of 2.0% for 1 hour of exposure.

Disinfection of test objects contaminated with mycobacteria was detected when using orthophosphoric acid at a concentration of 1.5% after exposure for 24 hours and at a concentration of 2.0% after exposure from 1 hour, as well as nitric acid at a concentration of 2.0% after exposure of 5-24 hours.

A biological test on laboratory animals was the final stage in determining effective modes of application of inorganic acids for the devitalisation of mycobacteria. For this purpose, three groups of animals were formed, which were separately injected with a suspension obtained after treatment of test objects contaminated with the causative agent of tuberculosis *M. bovis* and the use of orthophosphoric acid (1.5%-24 hours) (experimental group I, n=12) and nitric acid (2.0%-5 hours) (experimental group II, n=12). The control (group III, n=10) was infected with a live pathogen of tuberculosis without the action of antimicrobial agents. The results of the experiment are presented in Table 4.

During a biological study of swabs from the test objects on laboratory animals, after their treatment with acids, the bactericidal properties of orthophosphoric acid at a concentration of 1.5% after a 24-hour exposure and nitric acid at a concentration of 2.0% after the exposure for 5 hours against the causative agent of tu-

Table 3. Bactericidal action of inorganic acids against M. bovis on test objects

Mode of the acid application		Test Object	Research results	
Concentration	Exposure		Experiment	Control
Orthophosphoric acid				
1.5%	1 hour	Wood	+	+
		Tile	+	+
		Batiste	+	+
		Glass	+	+
		Metal	+	+
	5 hours	Wood	+	+
		Tile	–	+
		Batiste	+	+
		Glass	–	+
		Metal	–	+
	24 hours	Wood	–	+
		Tile	–	+
		Batiste	–	+
		Glass	–	+
		Metal	–	+
2.0%	1 hour	Wood	–	+
		Tile	–	+
		Batiste	–	+
		Glass	–	+
		Metal	–	+
	5 hours	Wood	–	+
		Tile	–	+
		Batiste	–	+
		Glass	–	+
		Metal	–	+
	24 hours	Wood	–	+
		Tile	–	+
		Batiste	–	+
		Glass	–	+
		Metal	–	+
Nitric acid				
2.0%	1 hour	Wood	+	+
		Tile	–	+
		Batiste	+	+
		Glass	–	+
		Metal	–	+
	5 hours	Wood	–	+
		Tile	–	+
		Batiste	–	+
		Glass	–	+
		Metal	–	+
	24 hours	Wood	–	+
		Tile	–	+
		Batiste	–	+
		Glass	–	+
		Metal	–	+

Note: "–" – absence of mycobacteria growth; "+" – mycobacteria growth

Table 4. Results of biological study of the bactericidal properties of inorganic acids

Animal group	Research method		
	Allergic	Pathoanatomical	Cultural
I	–	–	–
II	–	–	–
III	+	+	+

Note: ˝–˝ – absence of tuberculosis infection; ˝+˝ – presence of tuberculosis infection

berculosis *M. bovis* were confirmed. Only the animals of the control group responded to the administration of tuberculin, and during the pathological examination, lesions characteristic of tuberculosis were found in those animals. In the pathological material taken from the experimental and control animals, *M. bovis* was isolated only from animals of the control group.

Disinfectants for use in veterinary medicine and animal husbandry are essential elements for controlling infectious agents, including zoonotic and antimicrobial-resistant microorganisms in managed animal species (Paliy et al., 2019; Rodionova et al., 2021; Wales et al., 2021).

The arsenal of modern disinfectants is quite limited, therefore the constant search and improvement of antimicrobial agents for the purpose of sanitation of livestock facilities is an urgent task for veterinary science and practice (Chen et al., 2021; Scollo et al., 2023). Traditional means used for the control and prevention of animal tuberculosis are aldehydes, chlorine derivatives, oxygen compounds, etc. (Yoo, 2018; Xiao et al., 2022). However, mycobacteria have developed increased resistance to most disinfectants and their use is ineffective in modern conditions.

Currently, due to the widespread use of disinfectants, especially due to the pronounced COVID-19 pandemic, resistance to disinfectants needs to be more clarified than antibiotics in this field (Tarashi et al., 2022).

To destroy tuberculosis pathogens in the environment, we tested two inorganic acids whose effectiveness has been recognised in the practice of disinfection (Ganesh et al., 2012; Didouh et al., 2022). These inorganic acids are used in the dairy industry (Ózsvári and Ivanyos, 2022) and in water sanitation (Gul et al., 2021).

During the study, we found that different types of mycobacteria have varying sensitivity to chemical compounds. For instance, *M. fortuitum*, a fast-growing atypical mycobacteria, was most resistant, while *M. bovis*, the tuberculosis pathogen, was the least. Other researchers have also noted the high resistance of atypical mycobacteria to antimicrobial agents (Le Dantec et al., 2002; Chang et al., 2015). Moghaddam et al. (2022) showed a significant difference in resistance between *M. fortuitum* and other atypical mycobacteria.

Dauendorffer et al. (2000) demonstrated that *M. xenopi* is more resistant to disinfectants than *M. tuberculosis* or *M. smegmatis* and suggested that strains from the environment may be more resistantto disinfectants than strains isolated from pathological samples.

A biological test is mandatory to study the bactericidal properties of antimicrobial agents. Guinea pigs are the most sensitive model for anti-tuberculosis bioassay (Clark et al., 2014), and we considered this in our studies.

It is recommended that protocols for the use of disinfectants be tested under the specific conditions required to ensure their suitability for use (Uy et al., 2022).

The development of new inorganic acid-based disinfectants and their direct testing in production conditions is a prospect for further research.

Conclusions

Bacteriological research has shown that orthophosphoric acid can destroy atypical mycobacteria *M. fortuitum* in solution, and the causative agents of tuberculosis *M. bovis*, when applied to test objects at a concentration of 1.5% for 24 hours. Nitric acid also exhibited tuberculocidal properties at a concentration of 2.0% at exposure of 5 hours. It has been shown that atypical mycobacteria *M. fortuitum* species are more resistant to the action of inorganic acids, compared to the causative agents of tuberculosis *M. bovis* and *M. avium*. Orthophosphoric and nitric acids can be used for preventive and forced disinfection in cases of tuberculosis in farm animals.

Currently, most disinfectants offered by manufacturers are ineffective against mycobacteria. As a result, we urgently need to search for new and improved disinfectants with tuberculocidal properties. Our research results broaden the range of anti-tuberculosis disinfectants, enabling us to rationally rotate disinfectants in the general complex of veterinary and sanitary measures.

References

1. BALANI, K. A., T. R. SAHASRABUDHE, K. MEHTA and S. MIRZA (2023): TB patients: Is sputum disinfection important? Indian J Tuberc. 70, 142-146. 10.1016/j.ijtb.2022.03.027
2. BOCIAN, E., W. GRZYBOWSKA and S. TYSKI (2014): Evaluation of mycobactericidal activity of selected chemical disinfectants and antiseptics according to European standards. Med. Sci. Monit. 20, 666-673. 10.12659/MSM.890175
3. BOISSON-DUPUIS, S. (2020): The monogenic basis of human tuberculosis. Hum Genet. 139, 1001-1009. 10.1007/s00439-020-02126-6
4. BORHAM, M., A. OREIBY, A. EL-GEDAWY, Y. HEGAZY, H. O. KHALIFA, M. AL-GAABARY and T. MATSUMOTO (2022): Review on bovine tuberculosis: An emerging disease associated with multidrug-resistant mycobacterium species. Pathogens 11, 715. 10.3390/pathogens11070715
5. BOUCHEZ-ZACRIA, M., A. COURCOUL and B. DURAND (2018): The distribution of bovine tuberculosis in cattle farms is linked to cattle trade and badger-mediated contact networks in south-western France, 2007-2015. Front. Vet. Sci. 5, 173. 10.3389/fvets.2018.00173
6. BYRNE, A. W., D. BARRETT, P. BRESLIN, J. FANNING, M. CASEY, J. M. MADDEN, S. LESELLIER and E. GORMLEY (2022): Bovine tuberculosis in youngstock cattle: A narrative review. Front. Vet. Sci. 9, 1000124. 10.3389/fvets.2022.1000124
7. CHANG, C. T., E. G. COLICINO, E. J. DIPAOLA, H. J. AL-HASNAWI and C. M. WHIPPS (2015): Evaluating the effectiveness of common disinfectants at preventing the propagation of Mycobacterium spp. isolated from zebrafish. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 178, 45-50. 10.1016/j.cbpc.2015.09.008
8. CHEN, N., P. QIN, Y. LIU, Y. YANG, H. WEN, L. JIA, J. LI and Z. ZHU (2021): Influence of new compound disinfectant from n-dodecyl-2-(piridin-1-ium) acetamide chloride on pathogenic microorganisms in poultry houses. Front. Microbiol. 12, 735859. 10.3389/fmicb.2021.735859
9. CLARK, S., Y. HALL and A. WILLIAMS (2014): Animal models of tuberculosis: Guinea pigs. Cold Spring Harb Perspect Med. 5, a018572. 10.1101/cshperspect.a018572
10. DAUENDORFFER, J. N., C. LAURAIN, M. WEBER and M. DAILLOUX (2000): Evaluation of the bactericidal efficiency of a 2% alkaline glutaraldehyde solution on Mycobacterium xenopi. J. Hosp. Infect. 46, 73-76. 10.1053/jhin.2000.0793
11. DIDOUH, N., N. BENDIMERED, F. POSTELLEC, E. DEPERIEUX, I. LEGUERINEL and B. M. BOUDJEMÂA (2022): Effect of hydrophobic or hydrophilic characteristics of B. cereus spores on their resistance to detergents. J. Food Prot. 85, 706-711. 10.4315/JFP-21-286.

12. GANESH, V., N. S. HETTIARACHCHY, C. L. GRIFFIS, E. M. MARTIN and S. C. RICKE (2012): Electrostatic spraying of food-grade organic and inorganic acids and plant extracts to decontaminate Escherichia coli O157:H7 on spinach and iceberg lettuce. J. Food Sci. 77, M391-396. 10.1111/j.1750-3841.2012.02719.x.
13. GHANBARI, M. K., H. A. GORJI, M. BEHZADIFAR, A. SHOGHLI and M. MARTINI (2022): Strategic planning, components and evolution in zoonotic diseases frameworks: one health approach and public health ethics. J. Prev. Med. Hyg. 62, E981–E987. 10.15167/2421-4248/jpmh2021.62.4.2323
14. GUL, A., J. HRUZA and F. YALCINKAYA (2021): Fouling and chemical cleaning of microfiltration membranes: A mini-review. Polymers (Basel) 13, 846. 10.3390/polym13060846
15. LE DANTEC, C., J. P. DUGUET, A. MONTIEL, N. DUMOUTIER, S. DUBROU and V. VINCENT (2002): Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. Appl. Environ. Microbiol. 68, 1025-1032. 10.1128/AEM.68.3.1025-1032.2002
16. MATSUZAKI, S., K. AZUMA, X. LIN, M. KURAGANO, K. UWAI, S. YAMANAKA and K. TOKURAKU (2021): Farm use of calcium hydroxide as an effective barrier against pathogens. Sci. Rep. 11, 7941. 10.1038/s41598-021-86796-w
17. MIGLIORI, G. B., C. W. M. ONG, L. PETRONE, L. D'AMBROSIO, R. CENTIS and D. GOLETTI (2021): The definition of tuberculosis infection based on the spectrum of tuberculosis disease. Breathe (Sheff) 17, 210079. 10.1183/20734735.0079-2021
18. MOGHADDAM, S., F. NOJOOMI, A. DABBAGH MOGHADDAM, M. MOHAMMADIMEHR, F. SAKHAEE, M. MASOUMI, S. D. SIADAT and A. FATEH (2022): Isolation of nontuberculous mycobacteria species from different water sources: a study of six hospitals in Tehran, Iran. BMC Microbiol. 22, 261. 10.1186/s12866-022-02674-z
19. MOHAMED, A. (2019): Bovine tuberculosis at the human-livestock-wildlife interface and its control through one health approach in the Ethiopian Somali Pastoralists: A review. One Health 9, 100113. 10.1016/j.onehlt.2019.100113
20. ÓZSVÁRI, L. and D. IVANYOS (2022): The use of teat disinfectants and milking machine cleaning products in commercial Holstein-Friesian farms. Front. Vet. Sci. 9, 956843. 10.3389/fvets.2022.956843
21. PALIY, A., N. SUMAKOVA, R. PETROV, O. SHKROMADA, L. ULKO and A. PALII (2019): Contamination of urbanized territories with eggs of helmiths of animals. Biosystems Diversity 27, 118-124. 10.15421/011916
22. RODIONOVA, K., A. PALIY and M. KHIMYCH (2021): Veterinary and sanitary assessment and disinfection of refrigerator chambers of meat processing enterprises. Potravinarstvo Slovak Journal of Food Sciences. 15, 616-626. 10.5219/1628
23. SANDER, V. A., E. F. SÁNCHEZ LÓPEZ, L. MENDOZA MORALES, V. A. RAMOS DUARTE, M. G. CORIGLIANO and M. CLEMENTE (2020): Use of veterinary vaccines for livestock as a strategy to control foodborne parasitic diseases. Front. Cell Infect. Microbiol. 10, 288. 10.3389/fcimb.2020.00288
24. SAXENA, S., H. P. SPAINK and G. FORN-CUNÍ (2021): Drug resistance in nontuberculous mycobacteria: Mechanisms and models. Biology (Basel) 10, 96. 10.3390/biology10020096
25. SCOLLO, A., A. PERRUCCI, M. C. STELLA, P. FERRARI, P. ROBINO and P. NEBBIA (2023): Biosecurity and hygiene procedures in pig farms: Effects of a tailor-made approach as monitored by environmental samples. Animals (Basel) 13, 1262. 10.3390/ani13071262
26. SEDIGHI, T. and L. VARGA (2021): Evaluating the bovine tuberculosis eradication mechanism and its risk factors in England's cattle farms. Int. J. Environ. Res. Public Health 18, 3451. 10.3390/ijerph18073451
27. SHINODA, N., S. MITARAI, E. SUZUKI and M. WATANABE (2016): Disinfectant-susceptibility of multi-drug-resistant Mycobacterium tuberculosis isolated in Japan. Antimicrob. Resist. Infect. Control 5, 3. 10.1186/s13756-016-0102-y
28. SIMMONDS, R. C. (2017): Chapter 4. Bioethics and animal use in programs of research, teaching, and testing. In: Weichbrod, R. H., Thompson, G. A. H., Norton, J. N. (Eds.). Management of animal care and use programs in research, education, and testing. 2nd edition. CRC Press, Taylor & Francis, Boca Raton. Pp. 1-28. 10.1201/9781315152189-4
29. TARASHI, S., S. D. SIADAT and A. FATEH (2022): Nontuberculous mycobacterial resistance to antibiotics and disinfectants: Challenges still ahead. Biomed. Res. Int. 2022, 8168750. 10.1155/2022/8168750
30. TURGENBAYEV, K. A., A. M. BORSYNBAYEVA, A. A. PLAZUN and R. K. TURGENBAYEV (2021): Tuberculosis prevalence in animals and humans in the Republic of Kazakhstan. Vet. World. 14, 2362-2370. 10.14202/vetworld.2021.2362-2370
31. UY, B., H. READ, S. VAN DE PAS, R. MARNANE, F. CASU, S. SWIFT and S. WILES (2022): The efficacy of commercial decontamination agents differs between standardised test settings and research laboratory usage for a variety of bacterial species. Peer J. 10, e13646. 10.7717/peerj.13646
32. WALES, A. D., R. J. GOSLING, H. L. BARE and R. H. DAVIES (2021): Disinfectant testing for veterinary and agricultural applications: A review. Zoonoses Public Health 68, 361-375. 10.1111/zph.12830
33. XIAO, S., Z. YUAN and Y. HUANG (2022): Disinfectants against SARS-CoV-2: A Review. Viruses 14, 1721. 10.3390/v14081721
34. YOO, J. H. (2018): Review of disinfection and sterilization - back to the basics. Infect. Chemother. 50, 101-109. 10.3947/ic.2018.50.2.101
35. ZAVGORODNII, A. I., S. A. POZMOGOVA, M. V. KALASHNYK, A. P. PALIY, L. V. PLYUTA and A. P. PALII (2021): Etiological factors in triggering non-specific allergic reactions to tuberculin in cattle. Regulatory Mechanisms in Biosystems 12, 228-233. 10.15421/022131

36. ZHANG, H., M. LIU, W. FAN, S. SUN and X. FAN (2022): The impact of *Mycobacterium tuberculosis* complex in the environment on one health approach. Front. Public Health 10, 994745. 10.3389/fpubh.2022.994745

Baktericidna svojstva nekih organskih kiselina u odnosu na mikobakterije

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Danas, unatoč uspjehu u borbi protiv životinjske tuberkuloze, još uvijek se u životinja javljaju sporadični slučajevi infekcije s uzročnicima bolesti i s atipičnim mikobakterijama. Zbog činjenice da mikobakteriozu životinja nije moguće liječiti lijekovima, prvo mjesto za njihovu prevenciju i eliminaciju pripada nespecifičnim mjerama koje uključuju dezinfekciju. Jedinstvena struktura stanica mikobakterija određuje njihovu visoku otpornost na antimikrobna sredstva, stoga je stalna potraga za učinkovitim dezinfekcijskim sredstvima hitan zadatak veterinarske znanosti. Naš rad ima za cilj odrediti baktericidna svojstva nekih anorganskih kiselina u odnosu na mikobakterije. Provedeni su eksperimenti slijedeći moderne metodološke aspekte uporabom atipičnih mikobakterija *M. fortuitum*, patogena tuberkuloze *M. bovis* i *M. avium*. Ortofosforna (fosforna) kiselina (H_3PO_4) i dušična kiselina (HNO_3) su rabljene u eksperimentima. Sa suspenzijskom metodom istraživanja, otkriven je baktericidni učinak ortofosforne kiseline u odnosu na atipične mikobakterije *M. fortuitum* i patogen tuberkuloze *M. avium* kada je ispitana u koncentraciji od 1,5 % (24 sata) i 2,0 % (1-24 sata), protiv uzročnika tuberkuloze *M. bovis* u koncentraciji od 1,5 % (5-24 sata) i 2,0 % (1-24 sata). Dušična kiselina pokazala je baktericidni učinak kada je ispitana u otopini u odnosu na mikobakterije *M. fortuitum* i *M. avium* u koncentraciji od 2,0 % (5-24 sata) i u odnosu na *M. bovis* u koncentraciji od 2,0 % nakon izlaganja od 1 sata. Primijećena je različita razina intenziteta rasta mikobakterija u epruvetama nakon djelovanja anorganskih kiselina u subbaktericidnim i bakteriostatskim koncentracijama, što je izravno ovisilo o koncentraciji i izloženosti rabljenim kiselinama, kao i o vrsti ispitne kulture mikroorganizama. Kroz eksperimente je dokazano da anorganske kiseline mogu dezinficirati površine kontaminirane uzročnicima tuberkuloze. To se odnosi na različite površine poput: drva, pločica, tkanine, stakla i metala. Otkriveno je da je moguće rabiti ortofosfornu kiselinu (1,5 % tijekom 24 sata) i dušičnu kiselinu (2,0 % tijekom 5 sati) za dezinfekciju u slučajevima infekcije tuberkulozom. Ova otkrića potvrđena su biološkim testovima na laboratorijskim životinjama. Buduća istraživanja usredotočit će se na otkrivanje i razvoj novih lijekova sa snažnim baktericidnim svojstvima u odnosu na mikobakterije.

Ključne riječi: *ortofosforna kiselina, dušična kiselina, mikobakterija*